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THE EFFECT OF PYRIDOXINE AND PANTOTHENIC ACID DEFICIENCY ON SERUM IMMUNOGLOBULIN LEVELS IN HAMSTERS

by

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A Thesis Submitted to the Faculty of the Graduate School

of Loyola University in Partial Fulfillment of

the Requirements for the Degree of

Master of Science in

Oral Biology

May

1976

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DEDICATION

To my mother, Sylvia C. Davis, who by her encouragement in my pursuit of an education, is able to witness the completion of my formal studies.

ACKNOWLEDGMENTS

I wish to thank the members of my advisory committee: Dr. Anthony Garguilo, Dr. Patrick Toto and Dr. Gustav Rapp for their assistance and suggestions in writing this thesis. Their constructive criticisms have been most valuable.

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CHAPTER I

INTRODUCTION

Research in the past has dealt with the influence of nutritional factors upon resistance and susceptibility to infectious processes, (Robertson, 1934).[1]

Many studies since then have been concerned with nutrition in relation to acquired immunity, (Axelrod, 1968).[2] More recent investigations have implicated the role pyridoxine and pantothenic acid play in protein metabolism (Canham, 1969[3]; Roy and Axelrod, 1971[4]) and their influence in antibody formation (Axelrod and Pruzansky, 1955[5]; Axelrod, 1971[6]).

It is known that pyridoxine and pantothenic acid are involved in protein biosynthesis and in antibody formation. Most studies have investigated immunological response and host resistance in immunized animals on diets totally deficient in pyridoxine and/or pantothenic acid. It was decided that an investigation to ascertain blood serum levels of IgG immunoglobulin in unchallenged hamsters on a diet partially deficient in pyridoxine and pantothenic acid was in order.

CHAPTER II

LITERATURE REVIEW

A) Protein Biosynthesis Mediated by Pyridoxine and Pantothenic Acid

Vitamin B_6 was discovered in 1934 (Gyorgy, 1934)[7] and subsequently synthesized as pyridoxine. Bacterial studies later revealed the existence of two other natural forms of the vitamin, pyridoxal and pyridoxamine (Snell et al., 1942[8]; Snell, 1945[9]), collectively the compounds are referred to as Vitamin B_6. Pyridoxine, pyridoxamine and pyridoxal are converted through enzymatic pathways to pyridoxal -5'- phosphate which represents the co-enzyme form of Vitamin B_6 (McCormick et al., 1961[10]; Wada and Snell, 1961[4]). Pyridoxine in the co-enzyme form is concerned with a vast number of variety of enzyme systems associated with nitrogen metabolism (Snell, 1958[12]; Snell, 1972[13]). Early work by Schlenk and Snell (1945)[14] and Snell (1958)[12] have investigated the role pyridoxine plays in protein biosynthesis.

Some of the reactions catalyzed by pyridoxal -5'- phosphate include transamination, racemization, decarboxylation, cleavage, synthesis, dehydration and desulfhydration. The α

amino groups of many amino acids are removed by transamination (Snell, 1958)[12], which represents a major group of pyridoxal phosphate-catalyzed enzymes.

Binkley et al. (1952)[15] have shown that pyridoxine is required in the conversion of cysteine to pyruvic acid. Huovinen (1968)[16] showed that pyridoxal phosphate is an essential co-factor in enzymatic sulphhydration of serine in rat tissues.

Other areas of research were concerned with the role of pyridoxine in regulating amino acid absorption and transport within the small intestine. Munich (1965)[17] showed that pyridoxine is not an amino acid carrier of the rat small intestine; rather, the vitamin may be necessary to some metabolic process common to the transport systems. Christensen (1972)[18], in studying the nature of several distinct transport receptor sites of rat intestinal epithelium, concluded there was no direct evidence of pyridoxine involvement.

On the other hand, Asatoor et al. (1972)[19] have shown that in pyridoxine deficient rats, the rate of absorption of each of a group of 18 amino acids was depressed to about half its normal rate. They concluded that pyridoxine deficiency did not specifically affect active transport of free amino acid or dipeptide uptake by the enterocyte, but probably reduced the efficiency of efflux of free amino acids after transport had occurred. A failure in releasing amino acids from intestinal epithelial cells may reduce biosynthesis of proteins and essential enzyme systems needed for cellular metabolism vital to the host's survival.

Pantothenic acid was recognized in 1933 (Williams et al., 1933)[20] as a growth factor for yeast. It was later shown that pantothenic acid is converted via pantetheine to co-enzyme A (Baddiley, 1955)[21] which is an important catalyst of biological acetylation reactions (Lipmann et al., 1947[22]; Lipmann 1953[23]; Jaenicke and Lynen, 1960[24]). Co-enzyme A is enzymatically involved in acetylation of choline and certain aromatic amines and oxidation of fatty acids, pyruvate, ketoglutarate and acetaldehyde.

Only recently pantothenic acid has been implicated in protein biosynthesis. Lipmann (1971)[25] demonstrated that the analog between condensation in B-keto acid synthesis of carboxyl to methyl groups to yield $CO\text{-}CH_2$ and of carboxyl to amino groups to yield CO-NH in peptide synthesis was similar. He stated that 4'-phosphopantetheine in fatty acid synthesizing polyenzyme systems was also shown to take part in polypeptide synthesis.

Prescott and Vagelos (1972)[26] have shown that 4'-phosphopantetheine serves as a flexible arm that is utilized to transfer sequentially the growing peptide chain from one site to another.

Lee and Lipman (1974)[27] were able to purify a pantetheine-carrying protein fraction in yeast connected with growing peptide chains. This further substantiated the role of pantothenic acid in polypeptide synthesis.

The specific actions of pyridoxine and pantothenic acid in cell metabolism have led many investigators to study the effects of diets deficient in one or both of these vitamins on protein biosynthesis.

Montjar et al., (1965)[28] showed that a decreased incorporation of labeled orotic acid into the 29S and 18S components of ribosomal RNA and into messenger RNA was observed in the liver of pyridoxine deficient rats. The decreased synthesis of these components of RNA and the inability of the liver or spleen to incorporate labeled leucine and valine in vitro may provide a partial explanation of the mode of action of pyridoxine in protein biosynthesis.

Trakatellis and Axelrod (1965)[29] have demonstrated a decrease in number of cells/mg of spleen tissue in pyridoxine deficient rats, with a significant decrease in the DNA concentration/mg of this tissue. The administration of pyridoxine to deficient animals 24 hours before sacrifice effected an increase in number of cells, with a corresponding increase in DNA concentration/mg. In contrast, neither the number of cells nor DNA concentration/mg of liver tissue was affected by the deficiency. Bhagavan and Coursin (1971)[30] also observed no appreciable changes in the DNA, RNA and protein contents per unit weight of liver and brain of post-weanling rats deficient in pyridoxine.

Takami et al. (1968)[31] however, demonstrated that rats on a pyridoxine deficient diet for 60 days developed hepatic pyridoxal phosphate levels at 30% of the control values. They also pointed out that pyridoxal phosphate at a concentration as little as 2ug/gm of liver is sufficient for cell multiplication that follows partial hepatectomy. It was also found that in regenerating rat liver both nucleic acid and protein synthesis proceeded at the normal rate in a deficient state. This is in agreement with Trakatellis and Axelrod (1965)[29].

Holden et al. (1970)[32] investigated deficiencies of pantothenic acid and biotin on amino acid uptake by Lactobacillus plantarum. Their findings suggest that a reduction in lipid content makes the cell membrane unusually susceptible to distortion and may be responsible for the reduced level of amino acids intracellularly.

In other studies, Shinde and Ambegaokar (1971)[33] showed that animals fed a pantothenic acid deficient diet had a lower liver protein content then the controls. They believed that the function of this vitamin is confined more in the supernatant fraction of the liver than that of nuclear and mitochondrial fractions.

Roy and Axelrod (1971)[34] showed that <u>in vivo</u> incorporation of ^{14}C-amino acids into circulating serum albumin was diminished in pantothenic acid-deficient rats. No changes, however, were reported in liver polysomal profiles or the activities of the liver microsomal and soluble supernatant fractions in <u>in vitro</u> protein synthesis. They concluded that intracellular transport of newly synthesized proteins was impaired by a pantothenic acid deficient diet.

B) Antibody Structure and Synthesis

Tiselius and Kabat (1939)[35] demonstrated that the fraction of serum proteins, the γ globulins, which migrated slowest in electrophoresis, contained most of the serum antibodies. It has been only recently that amino acid sequence of human macroglobulins have been known. Cohen and Porter (1964)[36]; Edelman (1970)[39] and Putnam et al. (1971)[40] have done extensive work to elucidate antibody structure.

Takahaski et al. (1969)[41], using synchronized cell lines, showed that immunoglobulin synthesis is limited to late G, and early S portion of the cell cycle. It is during this cell phase that a dietary depression of pyridoxine and pantothenic acid may affect the amino acid metabolism necessary for immunoglobulin biosynthesis.

Sherr et al. (1971)[42] pointed out that secretory proteins are generally formed on polyribosomes attached to the endoplasmic reticulum, whereas non-exportable proteins are formed on free polyribosomes. His results indicate that in a cell-free system IgG was formed preferentially by the bound polyribosomes, although some free polyribosomes were also active in IgG synthesis. He further showed that IgG is transported to the Golgi complex by following labeled precursors of the IgG chain.

The role of immunoglobulins in host defense cannot be overstated. Specific metabolic effects from diets deficient in pyridoxine and/or pantothenic acid directly involve amino acid metabolism and polypeptide synthesis. Since immunoglobulins are protein in nature, any reduction or disturbance of protein metabolism may very well affect the animals' resistance and immunological response. Many investigators have been interested in studying the effects of pyridoxine and pantothenic acid deficiencies on immunological competence.

C) Pyridoxine and Pantothenic Acid Deficiencies on Immune Response.

Early research investigated diets deficient in B-complex, proteins and/or minerals. Cannon et al. (1943)[43], using rabbits, made no attempt to provide a balanced vitamin intake; however, he provided the animals with a protein deficient diet. He concluded that adult rabbits made hypoproteinemic produced antibodies less abundantly than did well-fed animals.

Stoerk and Zucker (1944)[44] using female albino rats, showed that complete or partial deficiency of riboflavin, pantothenic acid, thiamine or pyridoxine depresses thymus weight below that of controls of corresponding body weight. With a complete deficiency of any one factor there is a mild to marked atrophy of the thymus, which, however, may have been referable to losses in body weight.

Berry et al. (1945)[45] demonstrated that a decrease in total white blood cells (WBC), typhoid agglutinin titers, and polymorphonuclearcyte (PMN) phagocytic activity was observed in rats fed basal diets deficient in either/or casein, minerals or B vitamins. Ruchman (1946)[46] showed that mice on diets deficient of B-complex resulted in a somewhat decreased ability to produce neutralizing antibodies.

The finding that early pyridoxine and pantothenic acid deficiencies lower an animal's resistance when challenged by a specific antigen has been well documented in the literature. Stoerk and Eisen (1946)[47] immunized twenty-four male albino rats against washed sheep erythrocytes. It was apparent that animals fed a diet deficient in pyridoxine and then immunized exhibited little or no circulating antibodies. In a further study, Stoerk et al. (1947)[48] concluded that the only groups of rats having antibody titers lower than the controls were those completely deficient in protein or pyridoxine. Pantothenic acid deficiency failed to influence the antibody response to sheep red blood cells. Axelrod et al. (1947)[49] and Ludovici et al. (1951)[50] immunized rats on diets deficient in pyridoxine and pantothenic acid with washed human erythrocytes as the antigen. The results indicated impairment of antibody response in the pantothenic acid and pyridoxine deficient rats. Ludovici et al. (1949)[51] showed that rats after 3, 5, and 7 weeks on an experimental diet deficient in pantothenic acid had a decreased antibody response to immunized human erythrocytes.

Wertman and Sarandria (1951)[52] studied rats on various deficient diets and immunized the animals after six weeks with washed formalinized suspensions of Rickettsia typhi. They concluded that the ration containing one-tenth of the optimal level of vitamin B complex did not seriously interfere with circulating antibody production when a relatively large amount of antigenic material was injected. Pantothenic acid and thiamin deficiencies on the other hand influenced the antibody response when a relatively small amount of immunizing material was injected. Panda and Combs (1963)[53] showed that chicks fed diets partially deficient in either vitamin A, pantothenic acid, or riboflavin showed significantly lower agglutinin response to Salmonella pullorum as compared with controls.

Axelrod et al. (1961)[54] showed that skin hypersensitivity reactions of the early arthus-type (immediate hypersensitivity) were considerably reduced in pyridoxine-deficient guinea pigs, indicating a reduction in antibody levels.

Axelrod et al. (1962)[55] demonstrated that the delayed tuberculin hypersensitivity reaction in guinea pigs on a pyridoxine deficient diet was depressed. Davis (1974)[56] showed that congenital pyridoxine deficiency produces a defect in the capacity for expression of delayed hypersensitivity in 8 week old rats.

Robson and Schwarz (1975)[57,58] found that thoracic duct lymphocytes (TDL) from pyridoxine-deficient rats have a reduced capacity to respond to foreign lymphoid cells in the mixed lymphocyte reaction (MLR). This reduction in MLR activity by TDL cells reflected either a shift in the proportion of T and B cells in the TDL and/or an impairment in the capacity of such cells to function.

Hodges et al. (1962)[59,60], working with a small population of human subjects deficient in pantothenic acid or pyridoxine over a twelve week period, were unable to reveal sharply defined differences in the human immunologic response.

In a follow-up study, Hodges et al. (1962)[61] investigated human volunteers with a combined deficiency of pantothenic acid and pyridoxine, and immunized with tetanus, typhoid and polyvalent polio antigens. The results showed an insignificant rise in antibodies to typhoid H and tetanus, but an excellent response to polio virus. After restoration of the deficient vitamins, the response to tetanus and typhoid H antigens was excellent.

Harmon et al. (1963)[62], using pigs deficient in either pantothenic acid, pyridoxine or riboflavin over a three week study, showed decreased antibody titers to either human erythrocytes or other antigens. Following repletion, the pigs formerly deficient in B-vitamins had values of serum protein and antibody responses similar to the controls.

Woodruff (1970)[63], working with mice, found alterations of several host defense mechanisms in malnourished mice which were more susceptible to coxackievirus B_3 infection.

It becomes apparent that pyridoxine and pantothenic acid deficiencies modify host response by reducing antibody and lymphocyte levels to specific antigens. This has led other investigators to study vitamin deficiencies with regard to phagocytic activity and antibody synthesis.

Cottingham and Mills (1943)[64] showed that in every animal study a deficiency of any one vitamin sufficient to retard growth also caused a reduction in phagocytic activity of the white blood cells.

Klebanoff (1968)[65] reported a decreased killing of bacteria by phagocytes in homologous serum systems of the guinea pig on pyridoxine-deficient diets. The decreased rate of intracellular killing was correlated with a decrease in histochemically detectable myeloperoxidase. Bijsterveld (1971)[66] concluded that myeloperoxidase in intracellular digestion (phagocytosis) is pyridoxal-phosphate dependent, since the digestive capacity of pyridoxine-deficient phagocytes was reduced. Lederer et al. (1975)[67] pointed out that pantothenic acid deficiencies did not affect antigen metabolism or phagocytosis by the reticuloendothelial system.

Lucovici and Axelrod (1951)[68] noted that the process of antibody synthesis in animals is a more sensitive criterion of dietary adequacy than that of growth or symptomology.

Kumar and Axelrod (1968)[67], studying cellular antibody synthesis in rats given a pyridoxine-free diet, showed a decreased production of antibody-forming cells, which could account for the lowered level of circulating antibodies to sheep erythrocytes.

Axelrod (1971)[6] in a nutrition review stated that the most pronounced impairment of antibody response was in pantothenic acid, pyridoxine and pteroylglutamic acid deficiencies. The decreased serum hemolytic titer in pantothenic acid deficiency appears to be due to the decreased production of antibody forming cells, which does not seem to be related to an impairment in antigen metabolism.

Lederer et al. (1975)[67] have proposed that a decreased concentration of serum antibody in pantothenic acid deficient rats may reflect an impairment of antibody synthesis, a failure to secrete and properly distribute antibodies from their sites of formation into the blood, or a faulty antibody metabolism. Current information leads to the conclusion that pyridoxine and pantothenic acid function at different loci in the development of the immune response.

CHAPTER III

METHODS AND MATERIALS

Twenty-four dark ear albino male hamsters one month old (Whitney Farms, Aurora, N.Y.) were divided into two groups of twelve animals, one group serving as the control, the other serving as the experimental. Groups of six animals were housed in screen bottomed cages and given water ad libitum. Both animal groups were provided with a synthetic semi-purified rodent diet (Ralston Purina Co., St. Louis, Mo.) ad libitum (Table 1). However, the diet of the experimental group (#5820) contained only 40% of standard levels for pyridoxine hydrochloride and D-calcium pantothenate in the vitamin mix, other vitamin levels were the same as in control diet (#5810) (Table 2).

Table 1

Rodent Diets #5810* and #5820*

Casein	21.00%
Sucrose	15.00%
Dextrin	43.65%
Salka-Flox	3.00%
Crisco	10.00%
DL Methionine	.15%
Choline chloride	.02%
RP Mineral Mix	11.05%
* RP Vitamin Mix	2.00%
	100.00%

* Ralston Purina Co., St. Louis, Mo.

Table 2

Vitamin Mixture for Purified Diet #5810

And Related Diets

The vitamin mixture provides the following amount of vitamins per kilogram of diet:

	#5810	#5820
Thiamin hydrochloride	20 mg	
Riboflavin	20 mg	
Niacin	90 mg	
Pyridoxine hydrochloride	20 mg	8 mg
D-calcium pantothenate	60 mg	24 mg
Folic acid	4 mg	
D-biotin	.4 mg	
i-inositol	200 mg	
Vitamin B_{12} (in 0.1% trituration)	20 mcg	
Vitamin A acetate	40,000 IU	
Vitamin D_2	442 IU	
DL-alphatocopherol acetate	50 IU	
Menadione sodium bisulfite	20 mg	

All twenty-four hamsters were placed on the control diet (#5810) for one week. At the beginning of the second week twelve hamsters were given the experimental diet (#5820). The animals were checked regularly for any untoward reactions during the next three weeks. At the end of the experimental period the hamsters were sacrificed.

All animals were sedated by ether $(C_2H_5)O$, and the upper left quadrant of each animal was shaved. Then by sharp scapel dissection the axillary artery was exposed and nicked to allow pooling of the blood. Using sterile eye droppers, whole pooled blood was collected from each animal, placed in sterile test tubes, and then put in a cooler at 4°C. for 24 hours to allow a blood clot to precipitate. The serum fraction was decanted and centrifuged for 5 minutes at 4900 RPM. The serum fractions were then returned to the 4°C. cooler until serum immunoglobulin levels could be measured.

Serum immunoglobulin levels were measured quantitatively utilizing immunodiffusion plates (Microbiological Assoc., Bethesda, Maryland). Using 10 ul "dispo" micro pipets (Scientific Products, Evanston, Ill.) serum samples were placed in the plate wells until the serum was flush with the plate agar. Working standards, 10mg/ml, 5mg/ml, and 2.5mg/ml were plated in the same manner; however, 7 ul samples were transferred to the agar plates by transfer micropipets (Gilmont, Great Neck, N.Y.). Two determinations of each serum sample were done. The immunodiffusion plates were then incubated in a water-saturated atmosphere for 16-20 hours at 4°C. Diffusion rings were then measured by a Precision Viewer (Hyland Labs, Costa Mesa, Calif.). The data was analyzed by the T-test to determine any significant difference between the control and experimental groups.

CHAPTER IV

RESULTS

The experimental animals showed more of a tendency toward hyperirritability and hyperexcitability than did the controls. There were no fatalities in either group.

The disc diameters on the immunodiffusion plates were averaged for each animal (Table 3) then plotted against the known standards (Graphs 1 and 2). The corresponding IgG concentrations for each animal were determined (Table 4). The mean serum IgG concentration for the experimental animals was 5.0mg/ml and for the controls was 5.3mg/ml respectively. Statistical analysis using the "T" test indicated no significant difference between serum IgG concentration of the control or experimental groups, with a P 1/1,000 at α .05 level of confidence.

Table 3

<u>Collected Data</u>

Standards mg/ml	Controls X_1	Disc Diameter (mm) 1	Disc Diameter (mm) 2	Average Disc Diameter	Experimental X_2	Disc Diameter (mm) 1	Disc Diameter (mm) 2	Average Disc Diameter
10.0		13.0	12.9	13.0		13.0	12.9	13.0
5.0		11.0	11.1	11.1		11.5	11.5	11.5
2.5		9.0	9.4	9.2		9.5	9.5	9.5
	1	11.2	10.5	10.9	13	10.4	11.5	11.0
	2	11.0	11.2	11.1	14	11.0	11.5	11.3
	3	11.0	11.1	11.1	15	11.6	11.5	11.6
	4	11.0	11.0	11.0	16	11.5	11.7	11.6
	5	11.6	10.7	11.2	17	11.6	11.6	11.6
	6	11.4	10.5	11.0	18	11.2	11.5	11.4
	7	11.7	11.4	11.6	19	11.4	11.7	11.6

Table 3

Collected Data
(con't.)

Standards mg/ml	Controls X_1	Disc Diameter (mm) 1	Disc Diameter (mm) 2	Average Disc Diameter	Experimental X_2	Disc Diameter (mm) 1	Disc Diameter (mm) 2	Average Disc Diameter
	8	10.0	10.7	10.4	20	11.9	11.6	11.8
	9	11.5	10.9	11.2	21	11.5	11.1	11.3
	10	12.1	12.0	12.1	22	10.8	11.7	11.3
	11	11.5	11.4	11.5	23	12.0	11.4	11.7
	12	11.0	11.2	11.1	24	11.4	11.6	11.5

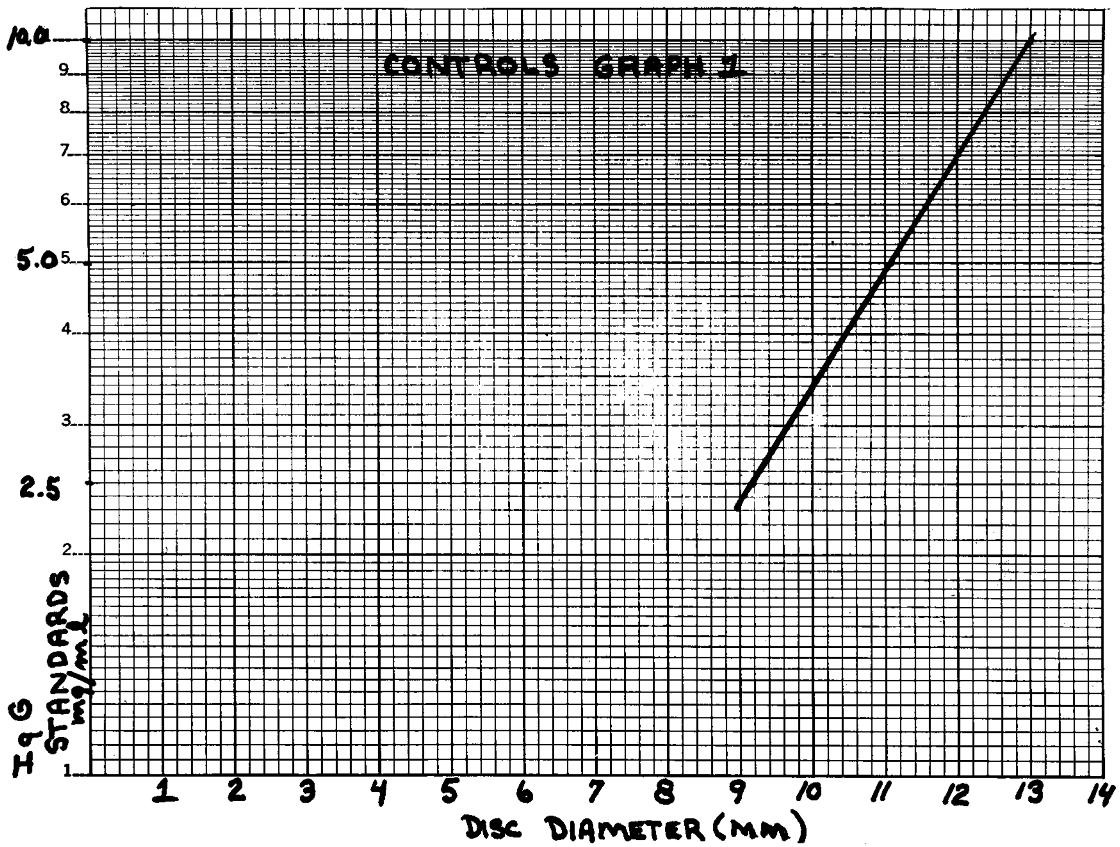
Table 4

Unknown IgG Concentrations of

Experimental and Control Animals

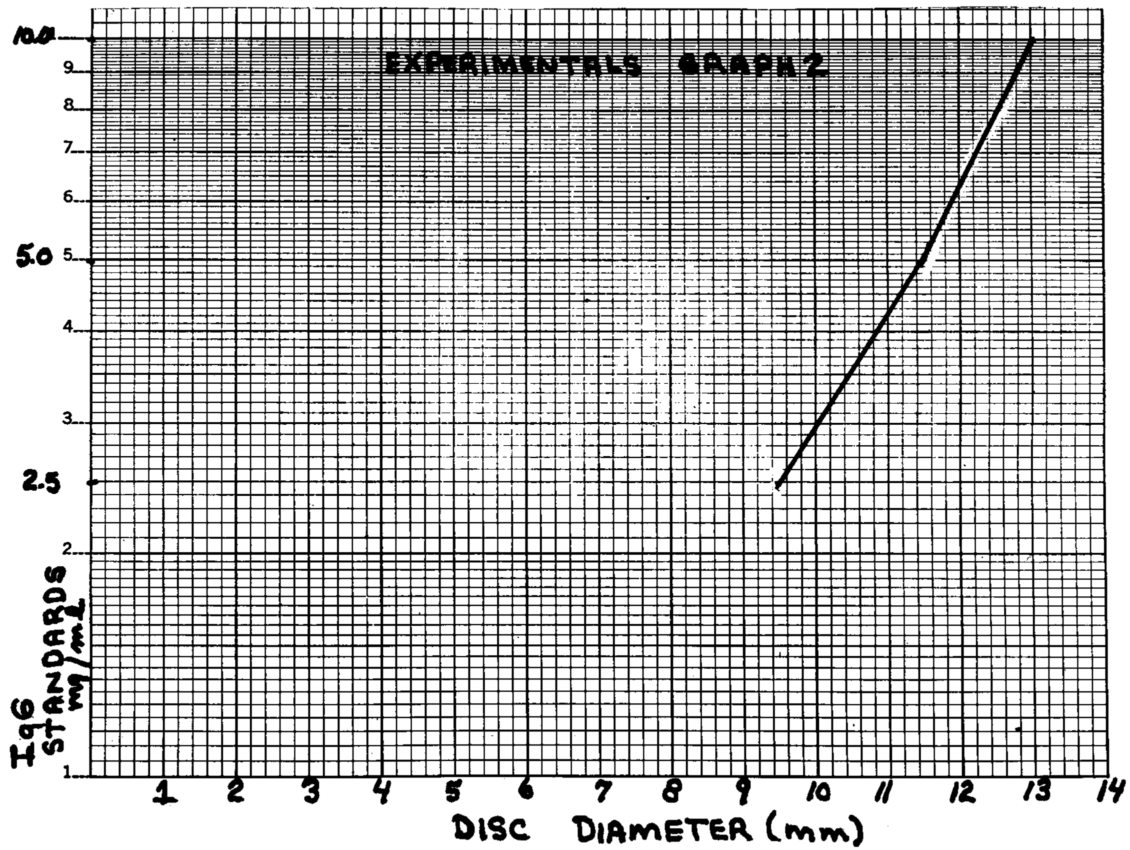
	Controls X_1	mg/ml	Experimentals X_2	mg/ml
	1	4.7	13	4.2
	2	5.0	14	4.6
	3	5.0	15	5.3
	4	4.9	16	5.3
	5	5.3	17	5.3
	6	4.9	18	4.8
	7	6.5	19	5.3
	8	3.9	20	5.8
	9	5.3	21	4.6
	10	7.3	22	4.6
	11	5.9	23	5.5
	12	5.0	24	5.0
Mean		5.3		5.0
SD		.89		.47
"T"		.976	-207 > t > 2.07	

P $<$ 1/1000



Graph I

Reference line plotted from working standard IgG serum dilutions -- control group.



Graph 2

Reference line plotted from working standard IgG serum dilutions -- experimental group.

CHAPTER V

DISCUSSION

In this study the radial diffusion type of precipiten reaction in agar has been found to be a useful means for estimating the quantity of immunoglobulins present in various systemic fluids (Mancini et al., 1965[70]; Fahey and McKelvey, 1965[71]; Vaerman et al., 1969[72]). Application of this procedure for the assay of immunoglobulins provides data of clinical importance for many diseases (Stiehm and Fudenberg, 1966[73]; Perry et al., 1974[74]).

The single radial diffusion method is, therefore, suitable for very accurate quantitative determinations, without any resort to end-point methods and the interpolations they require. It is assumed that the results obtained give an accurate indication of the level of protein synthesis.

Antibodies are considered to be highly specialized proteins. In this study we are mainly dealing with the mechanisms of protein biosynthesis and the reported role of pyridoxine and pantothenic acid.

Previous research has related animal diets which were completely deficient in pyridoxine and pantothenic acid, to impairment of protein biosynthesis and metabolism, reduction of circulating antibodies, and altered cell mediated hypersensitivity. These studies have reflected severe dietary deficiencies on host response. Axelrod et al. (1961)[54] reported high mortality rates in guinea pigs on diets totally deficient in pyridoxine and pantothenic acid. In the present study no animals succumbed while on partially depleted diets, and, therefore, could be considered healthy.

A state of inanition always accompanies the deficiencies under consideration (Axelrod and Pruzansky, 1955[5]). Experimental data indicate that host response of inanition controls, both of the "pair-fed" and "paired-weighed" type, is not impaired (Axelrod and Pruzansky, 1955[5]; Trakatellis and Axelrod, 1964[75]). This argues against any significant role of inantion per se and strongly suggests that observed effects in the deficiency state are specific for the vitamin in question.

In nearly all the studies using specific dietary depletions, normal antibody titers were found in the inanition controls (Axelrod et al., 1947[49]; Axelrod and Pruzansky, 1955[5]; Kumar and Axelrod, 1968[69]) that is, those animals maintained by the control of the quantity of food intake at the body weight of the specifically deprived animals. Woodruff (1970)[63] however, found that inanition caused involution of the lymphoid tissues in mice which adversely influenced antibody production. This could be expected when animals are under stressful dietary conditions. Without adequate nutrition, cellular functions are not at optimum levels.

It is known that pyridoxine deficiency (Sebrell and Harris, 1968[76]) is also characterized by anemia, dermatitis, growth retardation and alteration in neuronal function, including neuropathies, hyperirritability, hyperexcitability and convulsions. Pantothenic acid deficiencies (Follis, 1958[77]; Williams, 1973[78]) are characterized by growth retardation, dermatitis and impaired reproduction.

When animals are completely deprived of needed nutrients, severe metabolic states result. In completely pyridoxine-deficient rats and mice, an increased excretion of urea and elevated blood urea levels were observed, indicating an increased tendency of oxidation of amino acids for energy (Hawkins et al., 1959[79]).

Evidence also exists that severe pyridoxine deficiency causes an increase of urinary excretion of endogenous oxalate in rats (Gershoff and Faragalla, 1959[80]).

The finding that hyperoxaluria can be enhanced by administration of glycine indicates that an alteration in its metabolism may be involved, and that in a deficiency state, more glycine is metabolized via glyoxylic acid to oxalic acid than in normal animals.

Glycine and serine are important precursors in purine synthesis. It might be expected that a complete pyridoxine deficiency would affect purine formation by reducing DNA and RNA levels, hence limiting cellular reproduction and protein synthesis.

It is also established that pyridoxine depletion has serious implications in tryptophan metabolism (Korbitz et al., 1963[81]). Pyridoxine deficient rats excreted abnormally large quantities of kynurenine, hydroxykynurenine and xanthurenic acid as a result of incomplete tryptophan metabolism.

It is conceivable that with reduced levels of pyridoxine and pantothenic acid, these vitamins may have been directed towards more important cellular functions, like immuno-defense mechanisms. It has been suggested that pyridoxine deficiency in rats resulted in an increase in the activity of an enzyme which inactivates pyridoxal phosphate dependent apoenzymes (Nutr. Rev., 1972[82]). The activity of this enzyme increased in intestine and skeletal muscle, but not in liver and heart, and may function to release pyridoxal phosphate for more vital functions. This may well explain why the serum IgG levels were similar in both control and experimental groups in the present study.

Another consideration, is that the length of time the animals were deprived may not have been long enough to measure any significant change in serum levels. It is noted that the serum IgG concentration of the experimental group was 5.0mg/ml versus 5.3mg/ml for the controls. In animals completely deprived of pantothenic acid and pyridoxine, serum antibody levels showed significant differences in some studies within three weeks (Ludovici et al., 1949[51]; Harmon et al., 1963[62]).

The present study suggests that substantial levels of pyridoxine and pantothenic acid were available to maintain protein synthesis for the first three weeks, but if the experiment were allowed to continue, protein synthesis may have been reduced to compensate for an inadequate vitamin intake. Further research, however, is needed to confirm this.

The results from the present study indicated that animals on dietary levels of pyridoxine and pantothenic acid at 40% of optimum, showed no significant difference in serum IgG concentration from that of control levels. The finding that immunoglobulin levels were similar in control and experimental animals, indicates that a partial depletion of pyridoxine and pantothenic acid in the diet does not have any influence on protein synthesis. The presumed suboptimal level of pyridoxine and pantothenic acid may not have been suboptimal, but at a sufficient level to maintain protein synthesis.

Takami et al., (1968)[31] pointed out that pyridoxal phosphate at a concentration as little as 2ug/g was sufficient for cellular response. It is apparent that the present research does not significantly alter the animals' nutritional state while animals whose diets are completely deficient have shown untoward side affects (Axelrod et al., 1961[54]; Sebrell and Harris, 1968[76]).

It is evident that the host response of the completely deprived animals is species specific. Human subjects deficient in pantothenic acid or pyridoxine did not reveal differences in immunologic response (Hodges et al., 1962[59,60]) as compared to Axelrod's experimental animals (Axelrod, 1971[6]). Lower phylogenetic levels have a tendency to be more sensitive to dietary disturbances than higher animal forms. The synthesis and turnover of nucleic acids and proteins is of great importance within and between species.

It is known that by using tritium labeled thymidine one can follow its incorporation into DNA (Cleaver, 1967[83]). If DNA can be reabsorbed and reutilized by other cells after cell cytolysis (Toto et al., 1969[84]) normal cellular metabolism could occur without interruption. This is based on the premise that reutilization of DNA and RNA allows available dietary levels of pyridoxine and pantothenic acid to be used more efficiently in synthesis of other cellular constituents, which may have been catabolized during cell lysis. As long as the animals were receiving pyridoxine and pantothenic acid in their diets. This was adequate to maintain synthesis of IgG antibodies.

The introduction of H^3Tdr as a specific label for DNA may be able to provide a tool for studying cell populations and DNA synthesis in vitamin deficient states of partially or completely deprived animals. Further investigation in this area is needed, however.

Other studies have shown that the production of circulating antibodies from the introduction of an antigenic stimulus was depressed in complete vitamin deficient states. In the present study, a partial depletion of pyridoxine and pantothenic acid in non-sensitized animals resulted in serum IgG levels which were similar for both control and experimental groups.

The administration of an antigen stimulates an intensive multiplication of host cells in certain organs concerned with immune responses (Cooper and Lawton, 1974[85]). Before a cell can be selected by an antigen it must be generated from an undifferentiated precursor and induced to manifest the appropriate antibody (Cooper and Lawton, 1974[85]). Immunologically competent B lymphocytes are stimulated by contact with an antigen to proliferate and thereby form either plasma cells which synthesize antibody or additional B lymphocytes called memory cells (Cooper and Lawton, 1974[85]). The production of memory cells is a mechanism for the expansion of selected clones, enabling an animal who has been exposed to an antigen once, to respond more promptly.

It is known that pyridoxine is involved with amino acid metabolism and protein synthesis. Pantothenic acid is the basis of interrelationships between the Embden-Meyerhof pathway and the Krebs Cycle. The formation of acetyl COA (active acetate) which contains pantothenic acid, is a major junction point which integrates carbohydrate, fat and protein metabolism. In animals completely deficient in pyridoxine and/or pantothenic acid essential enzyme systems may be altered which result in the inability of the B lymphocytes to proliferate (Cooper et al., 1973[86]), of the plasma cell to synthesize the appropriate antibody (Gasser and Silvers, 1974[87]; Baumal and Scharff, 1973[88]) or in the production of memory cells needed by the animal to respond to a second antigenic challenge. Another possibility, is that distortion of the cell membrane may result in the inability to secrete newly synthesized proteins into the extracellular compartment (Axelrod, 1971)[6].

In the present study, a diet partially depleted in pyridoxine and pantothenic acid had no demonstrable affect on IgG synthesis. The animals were not challenged and had no immediate need for additional IgG antibodies. The available levels of pyridoxine and pantothenic acid were sufficient to maintain adequate levels of IgG in an unchallenged animal. The effect of a partial vitamin deficiency on serum IgG levels in challenged animals, using immunodiffusion techniques, has to be investigated.

If it is assumed that pantothenic acid and pyridoxine function at different loci within the cell, a partial dietary deficiency could allow other unknown pathways useful in protein metabolism to occur unhindered.

The idea that both vitamins are important in specific enzyme systems does not rule out the possibility that other enzymatic pathways, independent of pantothenic acid and pyridoxine, are utilized. The altered pathways may be of prime biosynthetic importance in partially deficient states. It was shown that in tryptophan metabolism, transamination of kynurenine and hydrorykyneurenine may involve two distinct enzymes with dissimilar characteristics (Brown et al., 1961)[89]. The excretion of xanthurenic acid in riboflavin and/or pyridoxine deficiency supports the contention that other enzymatic pathways are important (Charconnet-Harding et al., 1953)[90].

The function of enzyme systems in biological reactions is to decrease the initial energy of activation. It is possible that in the complete absence of pyridoxal and pantetheine, most biological reactions do not occur at any significant rate, because of the inability to reduce the energy of activation of the substrate. This may partially explain why in all complete vitamin deficiency studies one still encounters an antibody titer; however, it is significantly depressed. On the other hand, a partially depleted diet may be rate limiting due to the available levels of pyridoxine and pantothenic acid. It is quite evident that the rate of an enzyme reaction is directly proportional to the concentration of the enzyme; the more enzyme, the faster the reaction. The finding that IgG serum levels were similar may indicate that usual enzymatic pathways, even though reduced, along with altered pathways, may have been sufficient to maintain adequate IgG levels in the partially deprived animals.

An altered physical state will have a profound effect on enzyme and substrate concentrations, pH, ion concentration and hormonal activity. All these factors greatly influence enzyme action and may very well account for reduced antibody levels in animals which are totally deficient of pyridoxine and/or pantothenic acid.

CHAPTER VI

SUMMARY

A study was undertaken to quantitatively measure serum IgG levels in hamsters partially depleted in pyridoxine and pantothenic acid.

Twenty-four dark ear albino male hamsters, provided with a synthetic semipurified diet were divided into two groups of twelve animals, one group serving as the control, the other serving as the experimental. The diet of the Experimental group, however, contained pyridoxine hydrochloride and D. calcium pantothenate at 40% of standard levels. Three weeks after deprivation the hamsters were sedated and bled. Serum immunoglobulin levels were measured 48 hours later utilizing immunodiffusion techniques.

Results showed that mean serum IgG concentration of the experimental animals was 5.0mg/ml and for the controls was 5.3mg/ml respectively, indicating no significant difference.

Pyridoxine and pantothenic acid are required in the nutrition of all species of animals. Their role in metabolic processes is multiple and involves a large number of biochemical reactions. The finding that a partial dietary deficiency of

pyridoxine and pantothenic acid does not affect protein synthesis as found in complete vitamin deficiency states has been discussed.

CHAPTER VII

CONCLUSIONS

1) The radial diffusion technique employed in this study gave an accurate quantitative determination of the level of protein synthesis.

2) Animals who are completely deficient in pyridoxine and/or pantothenic acid develop severe "nutritional syndromes" which affects animal physiology.

3) Reduced levels of pyridoxine and pantothenic acid may have been directed toward more important cellular functions, like immuno-defense mechanisms.

4) The presumed suboptimal level of dietary pyridoxine and pantothenic acid may not have been suboptimal, but at a sufficient level to maintain protein synthesis.

5) Host response in the completely deprived animal is species specific.

6) Available dietary levels of pyridoxine and pantothenic acid may have been used more efficiently in cellular metabolism.

7) A partial dietary depletion of pyridoxine and pantothenic acid in an unchallenged animal had no demonstrable affect on IgG synthesis.

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5) Host response in the completely deprived animal is species specific.
6) Available dietary levels of pyridoxine and pantothenic acid may have been used more efficiently in cellular metabolism.
7) A partial dietary depletion of pyridoxine and pantothenic acid in an unchallenged animal had no demonstrable affect on IgG synthesis.

8) In a partial dietary deficiency other unknown pathways useful in protein metabolism may occur unhindered.

9) The similar level of IgG of both the control and experimental groups, may be related to the rate of enzyme reaction which are dependent on the available concentration of needed co-factors.

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APPENDIX

TABLE 1

STATISTICAL ANALYSIS

X_1		X_2		
ΣX_1	$= 63.7$	ΣX_2	$= 60.3$	$Hy = X_1 = X_2$
ΣX_1^2	$= 346.85$	ΣX_2^2	$= 305.41$	$df = 22$
$\overline{X}_1$	$= 5.308$	$\overline{X}_2$	$= 5.025$	$\alpha = .05$
S_1	$= 0.8898416$	S_2	$= 0.4673424$	$Sp = 0.711$
S_1^2	$= 0.7918181$	S_2^2	$= 0.218409$	

$$S = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}{N - 1}}$$

$$Sp^2 = \frac{(N_1 - 1)S_1^2 + (N_2 - 1)S_2^2}{N_1 + N_2 - 2}$$

$$\text{"t"} = \frac{\overline{X}_1 - \overline{X}_2}{Sp\sqrt{(1/N_1) + (1/N_2)}}$$

APPROVAL SHEET

The thesis submitted by Lowell B. Davis, D.D.S., ORAL BIOLOGY has been read and approved by the following Committee:

Dr. Anthony W. Garguilo, Clinical Professor; Chairman, Periodontics, Loyola.

Dr. Gustav W. Rapp, Administrative Advisor to the Dean; Professor; Chairman, Biochemistry, Loyola.

Dr. Patrick D. Toto, Professor, Chairman, Departments of General and Oral Pathology Coordinator of Advanced Education, Loyola.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of MASTERS OF SCIENCE.

May 14 1976
Date

Patrick D. Toto
Director's Signature